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EFFECTS OF DIETARY RESTRICTION AND EXERCISE ON THE AGE-RELATED PATHOLOGY OF THE RAT

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ABSTRACT

Intervention of the aging process is an effective, experimental means of uncovering the bases of aging. The most efficacious and commonly used intervention used to retard the aging processes is dietary restriction (DR). It increases mean and maximum life spans, delays the appearance, frequency, and severity of many age-related diseases, and more importantly, attenuates much of the physiological decline associated with age. Although the subject of intense research, the mechanism by which DR alters the aging processes is still unknown. Physical exercise is another effective intervention shown to affect aging phenomena, especially when applied in combination with DR. Mild exercise in concert with DR is beneficial, but vigorous exercise coupled with DR could be deleterious. With regard to pathology, exercise generally exerts a salutary influence on age-related diseases, both neoplastic and non-neoplastic, and this effect may contribute to the increase in median life span seen with exercised rats. Exercise coupled with 40% DR was found to suppress the incidence of fatal neoplastic disease compared to the sedentary DR group. Exercise with mild DR suppressed the incidence of multiple fatal disease and chronic nephropathy, and also delayed the occurrence of many age-related lesions compared to the ad libitum (AL) control group. However, these effects may have little bearing on the aging process per se, as maximum life span is only minimally affected. Although not as intensively studied as DR, results from studies that utilize exercise as a research probe, either alone or in combination with DR, have helped to assess the validity of proposed mechanisms for DR and aging itself. Neither the retardation of growth rate nor the increase in physical activity, observed with either exercise or DR, appear to contribute to the anti-aging action of DR. Moreover, results from lifelong exercise studies indicate that the effects of DR do not depend upon changes in energy availability or metabolic rate. The mechanisms involving effects on adiposity or immune function are also inadequate explanations for the action of DR on aging. Of the proposed mechanisms, only one, as postulated by the Oxidative Stress Hypothesis of Aging, tenably accounts for the known effects of DR and exercise on aging.

KEY WORDS

Calories, Food intake, Physical activity, Longevity, Life span, Aging, Disease

INTRODUCTION

Interventions of the aging processes by various experimental manipulations provide gerontologists with the opportunity to examine the basic mechanisms underlying aging processes. In experimental gerontology, dietary restriction (DR) represents the most effective and well-known intervention by which to explore aging. DR increases both median and maximum life spans, delays the appearance, frequency, and severity of many age-related diseases, and attenuates much of the physiological decline associated with aging (1-5).

In studies involving changes in median and maximum life spans, DR is generally considered the gold standard against which the results from other interventions are gauged.

Another intervention, physical exercise, has recently received attention as a method of altering aging. Although not as robust as DR, exercise has been reported to extend life span and alter the age-related development of pathology and functional deterioration (6-8). The available evidence suggests that, in addition to attaining the same end, viz., intervention of the aging processes, these two experimental manipulations may also utilize certain common mechanisms to attain that end.

Interestingly, exercise and DR share a number of common characteristics. Both involve heightened physical activity. Exercise is, by definition, a subset of physical activity with structured and sustained musculoskeletal movements. With DR, high levels of physical activity arise spontaneously and are sustained throughout the life span (5). In addition, animals from both groups exhibit similar metabolic profiles. Many voluntary exercised rats do not increase their food intake enough to compensate for the increased energy expenditure. Consequently, similar to DR rats, voluntary exercised rats have lower body weights compared to sedentary controls (9,10). Endurance exercise modulates energy utilization in a manner similar to that of long-term DR. For example, both DR and exercise training retard growth and prevent obesity (9,11).

Retardation of the appearance and progression of disease are also characteristics shared by DR and exercise (1,10,12,13). To what extent DR and exercise

contribute to age-related pathology has not been fully assessed. Unlike measurements of life span, changes in the patterns and severity of age-related diseases have been under-appreciated as assessment tools for the actions of DR and exercise on aging processes. Few systematic studies have addressed the simultaneous effects of these interventions on aging processes in general, and on age-related diseases, in particular. This oversight is unfortunate because the analysis of age-related pathologies, as affected by DR and exercise, could provide us with clues to the underlying mechanism(s) of the aging processes and the pathogenesis of age-related diseases. The main purposes of this review are, (i) to present evidence from pathology that the anti-aging actions of DR and exercise are linked by common mechanisms, and (ii) to assess, in light of this evidence, the current mechanisms proposed to explain the actions of DR and exercise on the aging processes.

Metabolic Consideration of Dietary Restriction and Exercise

Since McCay's original finding (14) that DR increases longevity, gerontologists have tried to understand the mechanisms of aging by determining how DR works. Based on the metabolic characteristics observed in DR animals, several factors have been implicated in the life-extending effect of DR. Among these are the following: (i) the rate of growth as a potential key modulator of aging (14); (ii) the prevention of excess body fat accumulation (11); and (iii) changes in the energy utilization pattern from cell proliferation/reproduction to maintenance/repair pathways (2,15). Interestingly, these same metabolic characteristics are associated with exercise. Exercise, (i) retards the growth rate and reduces body weight (7), (ii) reduces the total-body fat content in young, growing rats (16), and (iii) limits the availability of energy for cell proliferation and growth in a manner similar to that of DR (9).

A commonly held view of DR action is that it retards the aging processes by reducing metabolic rate (17). This hypothesis is related to the "rate-of-living" theory of aging, which simply states that life span is inversely proportional to metabolic rate (18,19). A corollary of this hypothesis is that exercise, because of its elevated physical activity, should shorten life span. Although the subject is controversial, the most recent data do not lend support to this notion because the increase in longevity seen with DR is often associated with elevated physical activity (and presumably, an elevated metabolic rate). Yu et al. (5) noted that Fischer 344 rats subjected to DR (40% fewer calories than AL fed controls) maintained high levels of spontaneous activity throughout their life span in contrast to the AL fed controls.

Similarly, Holloszy and Schechtman (20) found that a decrease in calorie intake by 30% in male, Long-Evans rats elicited a much higher voluntary running activity over the life span than that observed in AL controls. The question arises, what role, if any, does elevated physical activity play in the anti-aging effects of DR? One possibility is that exercise per se (i.e., increased metabolic rate) is deleterious, but the associated changes (e.g., growth retardation and low fat accumulation) exert an anti-aging action that outweighs the effects of elevated metabolic rate. Another possibility is that exercise itself is not only not deleterious, but that it actually exerts a positive influence on longevity and age-related disease. These possibilities can only be addressed in studies in which both exercise and DR are examined simultaneously and their interactions tested.

Dietary Restriction, Exercise, and Longevity

In aging studies, population survival curves provide essential information on maximum and median life spans as well as the age-specific mortality of the population. Although the maximum life span of a population is commonly considered the more reliable index for aging processes, the survival curve (i.e., the mortality of the population) may be a better biomarker for age-specific morbidity, because it shows the onset and progression of age-related disease. In this review, we will first discuss the effects of DR and exercise on survival and longevity, then explore the relationship between these two interventions and age-related disease.

Exercise Studies in AL-fed Rats. As noted above, early studies utilizing exercise to intervene in aging processes had as their aim to test the "rate-of-living" theories of aging, most notably espoused by Rubner (19) and Pearl (18). Slonaker (21), and later Benedict and Sherman (22), showed that exercised animals had shorter life spans than did sedentary controls, results which supported the "rate-of-living" hypothesis. Unfortunately, the results of these studies are difficult to interpret, and the validity of their conclusions remains questionable. Both studies used small numbers of animals that were not housed under specific pathogen-free (SPF) conditions. It is well-known that the presence of infectious disease changes the characteristics of the survival curves, which can lead to false conclusions. The preponderant importance of using SPF conditions in survival studies has been clearly documented (23). Because proper precautions were not taken against infectious disease in early studies, the results of Slonaker (21) and Benedict and Sherman (22), which support the "rate-of-living" hypothesis, should be viewed with caution.

Later studies designed to test the effects of exercise on the aging processes revealed a life-extending action. Retzlaff et al. (8) showed that forced exercise significantly extended the life span in male and female Sprague-Dawley rats. Again, the validity of this study is also questioned because of its various peculiar outcomes. Among these, Goodrick (7) included: (i) the unusually shorter life spans of the control rats, (ii) similar life spans

for both male and female groups, and (iii) the heavier body weights of exercised rats, compared to their sedentary counterparts. In his own experiment, Goodrick (7) demonstrated that exercise exerted an anti-aging action in the AL rat.

Goodrick examined the effects of voluntary wheel-running initiated at 6 weeks of age on longevity in male and female Wistar rats (7). The median life spans of exercised male and female rats were 4 and 3 months longer, respectively, than sedentary controls. Exercise was also found to extend maximum life span. From these results, Goodrick (7) suggested that exercise might extend life span by slowing growth rate, a view proposed earlier by McCay et al. (14). Goodrick based his suggestion on the positive relation observed between growth duration and longevity, and the negative relation between growth rate and longevity. A major drawback of this study is the uncertainty concerning the housing conditions, especially the SPF status.

Recently, McCarter et al. (10) compared the effects of voluntary wheel exercise in an AL group to the responses of a sedentary control group, using SPF, male Fischer 344 rats. The exercised AL group ran substantially less than the exercised DR group, and their running activity decreased further with age. Throughout the life span these rats maintained a low (50-100 m/day) running activity. With regard to the spontaneous physical activity in the cage, no significant differences were observed between the exercised and sedentary groups. Thus, voluntary exercise was the only factor affecting energy expenditure between sedentary and exercised groups. Even with low running activity, the exercised AL group had a significantly lower body weight than the sedentary control group. Because exercised male rats do not increase food intake to compensate for the increase in energy expenditure (9,10), they do not gain the same weight as their sedentary AL counterparts. The survival curves for the sedentary and exercised AL groups were similar and not statistically different. The absence of an exercise-induced effect on longevity seen in the study of McCarter et al. (10) may be due to low voluntary wheel exercise activity.

Combined Effect of Exercise and Mild Dietary Restriction. In an attempt to circumvent the age-related decline in physical activity, Holloszy and colleagues (9) applied mild (8%) DR to the AL group. No differences in any parameter of interest were noted between this group and the traditional, sedentary AL rat. In SPF Long-Evans rats, 10-12 months old, a mild (8%) restriction in food take reversed the decrease in running, but did not prevent the gradual decline in the average distance run, from 6.4 km/24 h at age 9 months to 1.6 km/24 h at age 30 months. This exercised group had a significantly longer median life span than either the sedentary AL group or the pair-fed, sedentary control group. However, no extension of maximum life span was observed. A fourth group of rats, a paired-weight, sedentary group, was fed approximately 70% of the AL sedentary group's diet in order to maintain body weight at the level attained by the exercised group. This dietary regimen significantly extended both the average and the maximum life spans compared to the other three groups (9), confirming previous reports (4,5) on the effects of DR alone on longevity. The study by Holloszy et al. (9) demonstrated that although exercise accompanied by mild DR (8%) resulted in a decrease in growth (presumably due to the heightened energy expenditure), increased physical activity did not extend maximum life span. Thus, to the extent that changes in maximum life span signal alterations in the aging processes, these results suggest that exercise does not alter aging per se.

As discussed above, the results of McCarter et al. (10) studying the effects of exercise on AL fed rats are difficult to interpret because of the low exercise activity. To overcome this problem, a second study was undertaken in which voluntary exercise was coupled with mild (10%) DR, both of which were initiated at 6 weeks of age. In this study, a third DR group consisted of sedentary rats fed only 60% of the sedentary AL control's diet. Results from this study showed that both exercise with 10% DR and sedentary, 40% DR significantly extended median length of life and the age of the 10th percentile survivors, compared to the sedentary AL control. The life extending effects of exercise with 10% DR was less than that observed with 40% DR (24). It should also be noted that the increase in the 10th percentile survivors in the exercised with 10% DR group suggests that exercise does alter aging processes.

The basis for the discrepancy between the studies by Holloszy et al. (9) and McCarter et al. (24) has not been resolved at this time, but may involve differences in (i) the rat strain, (ii) the extent of calorie restriction, (iii) the degree of exercise, (iv) maintenance conditions, or (v) the age at which the exercise and DR were initiated. This last possibility rests on the presumption that the age at which the onset of the training regimen begins plays a significant role in the life expectancy of the rats (6,24). Regardless of the differences between the two studies, it is clear that exercise coupled with mild DR exerts some beneficial effects. The extent to which it alters longevity has yet to be unequivocally established.

Effects of Exercise on Dietary Restricted Rats. An important question arising from earlier work is whether exercise and DR act synergistically, antagonistically, or independently on the aging processes. In order to determine how enhanced physical activity contributes to the increased longevity elicited by DR, experiments must be performed with both interventions, applied singly and in combination. Goodrick and colleagues (25) studied the combined effects of exercise and every-other-day (EOD) feeding on longevity in male Wistar rats using voluntary wheel running (beginning at 6 weeks of age). Unexpectedly, this experiment produced effects opposite to those using exercise alone. Exercise increased the longevity of AL rats but de-

creased the longevity of EOD rats. The adverse effects of exercise on DR, non-barrier maintained Sprague-Dawley rats were also reported by Skalicky et al. (26). Many of these experiments are, unfortunately, marred by technical shortcomings: longevity studies were performed with very small numbers of animals (12-24 per group); food intake was not monitored; and the animals were not housed under barrier conditions. These shortcomings render somewhat ambiguous any conclusions drawn from these data.

More recently, Holloszy and Schechtman (20) examined the combined effects of exercise and DR on longevity of male rats. The study involved five groups, (i) sedentary with mild (8%) DR, (ii) exercise with 8% DR, (iii) exercise with 30% DR, (iv) sedentary with 30% DR (pair-fed group), and (v) sedentary with 46% DR, which was pair-weighted with the exercise with 30% DR group. The exercise with 30% DR group showed higher running activity and an extended maximum life span compared to the exercise with 8% DR. However, the exercise with 30% DR group was unable to extend maximum life span beyond that of either the pair-fed (sedentary with 30% DR) group or the pair-weighted (sedentary with 46% DR) group. Interestingly, the exercise with 30% DR group exhibited an increased mortality rate over the first 50% of their survival curve. Unfortunately, the spontaneous physical activity in each experimental group was not measured; thus, energy expenditure may not directly correlate to wheel running activity alone. Also, complete pathological profiles were not obtained on each of the rats in the various groups. The availability of such profiles would have been helpful, as a change in the age-related disease pattern is an important contributing factor to a change in the age-specific mortality rate. Notwithstanding these drawbacks, the results of Holloszy and Schechtman (20) like those of Goodrick et al. (25), suggest that exercise exerts deleterious effects on DR rats.

Somewhat different results were obtained in the study by McCarter et al. (10) and are discussed briefly above. In this study, 40% DR rats allowed to exercise exhibited an extended median life span, with no change in maximum or the 10th percentile survivorship. The reasons the results from this study differ from those described in the previous paragraph are unknown. Possible reasons include differences in (i) the rat strain used, (ii) the age at which exercise was initiated, (iii) the extent and type of DR, (iv) the severity of exercise, and (v) the housing and protection against specific pathogens.

Effects of Exercise in Female Rats and on Reduced Physical Activity. The female Long-Evans rat, unlike the male, increases its food intake in response to wheel exercise (27). In this study by Holloszy, the food intake of the exercising female was 37% greater than that of sedentary female rats up to age 10 months, and was 20% greater thereafter (27). Both sedentary and exercised groups showed almost the same peak body weights, although the exercised animals reached its peak more rapidly than the sedentary controls (27). Voluntary wheel exercise significantly extended median life span due to a later onset of mortality, but did not extend maximum life span. These findings are similar to those obtained in male Long-Evans rats with exercise and mild (8%) DR (9). Although hormonal conditions and age-related disease patterns differ between male and female animals, the results from this exercise study using females lend support to two conclusions. First, as suggested by Holloszy et al. (9), the effect of exercise on median life span in the AL group is not mediated by a decreased availability of energy for cell proliferation and growth. This conclusion is at variance with those of McCay et al. (14) and Goodrick (7) who felt that growth rate may be an important mechanism underlying the action of DR. Second, a large increase in food intake may not be harmful when balanced by an increase in energy expenditure.

Another approach to examining the effects of exercise on aging would be to restrict physical activity. In a study by Mlekush et al. (28), physical activity was decreased by housing mice in cages of different sizes and types. The results from the study showed that reduced physical activity decreased the median and maximal life spans of female Swiss-albino mice. Restriction of mobility was associated with a higher growth rate and a higher body weight despite a significant decrease in food intake. Notwithstanding food intake reduction and physical inactivity, mobility restriction still shortened the life span in these female mice, another finding that disputes the rate-of-living hypothesis originally proposed by Rubner et al. (19). Also, a major drawback to the study is that the possible effects of stress on longevity due to overcrowding were not addressed.

Conclusions. Consideration of the studies examined thus far suggests several general conclusions. First, an optimal combination of mild DR, exercise, and the age of exercise initiation may exert beneficial effects on the aging processes. Second, excessive exercise regimens coupled with DR appear to offer no additional benefits and may even be deleterious, depending on the animal's physiological condition. Third, physical activity and growth retardation do not seem to contribute importantly to the effect of DR; therefore, the idea that they may form the basis for the action of DR should be discarded. Finally, although exercise modulates metabolic characteristics, it seems that the effects of life-long exercise, and perhaps DR as well, may involve mechanisms other than changes in energy expenditure.

Dietary Restriction, Exercise, and Age-Related Disease

Pathological Analysis. The more frequent occurrence and increasing severity of pathological lesions, like physiological decline, appear to be inherently related to the aging processes. Many functional and structural changes take place with age in all organs. These changes are generally categorized as physiological

aging or pathological aging, depending on the degree of functional and/or morphological deterioration. Although the ideal animal model for studying physiological aging would be free of disease, the fact is, all animals develop diseases associated with aging. Thus, a tight link may exist between the physiological and pathological aging processes. If the occurrence or progression of certain types of diseases tightly correlate with physiological decline, the pathological analyses of the disease pattern may offer insight into the mechanisms underlying the aging processes. Similarly, any intervention of age-related pathologies, such as occurs with DR and exercise, may also offer insight into the mechanisms of aging. Unfortunately, too little information is available concerning the effects of exercise on age-related pathologies; thus, the opportunity to better understand the pathological process underlying the aging process has not been widely exploited.

Modulation of Disease Patterns by Exercise. In their earlier study, Holloszy et al. (9) reported that histopathological examination showed no significant differences in the causes of death among the voluntary exercise group, the sedentary AL group, and the sedentary, pair-fed animals. The sedentary DR controls, pair-weighed to the exercising rats, exhibited a markedly reduced incidence of neoplasms. Other than these observations, comprehensive pathological analyses were not provided. Consequently, the effects of exercise, alone or in combination with DR, on age-related disease patterns and the contribution of these changes in pathology on longevity remain unclear.

Recently, we examined the effects of diet and exercise on age-related disease (12,13). In particular, pathological analyses were performed to uncover correlations between the aging processes and the incidence and severity of major age-related diseases, especially those commonly observed at death. Tables 1 and 2 list the probable causes of death in all groups from two studies. Leukemia/lymphoma, pituitary tumor, chronic nephropathy, and cardiomyopathy are the major fatal diseases in the male Fischer 344 rat. In our first study, as noted in Table 1, the incidence of total neoplastic diseases at death was the same in the sedentary and exercised AL groups and in the sedentary DR group. However, the exercised DR group exhibited a significantly lower incidence of total neoplastic diseases compared to the other three groups. It should be emphasized that the data shown in Table 1 were obtained from animals that had died spontaneously. Thus, the sedentary AL group had the same incidence of neoplastic diseases as its DR counterpart, only because the AL rats died earlier from chronic nephropathy than the DR rats. The prevalence of fatal (severe) leukemia/lymphoma was not significantly different among all groups. However, DR was shown to delay the occurrence of this lesion in both sedentary and exercised rats. Although the incidence of fatal pituitary tumors was significantly higher in both the sedentary and exercised AL groups, exercise did not significantly affect the development of pituitary tumors in either the AL or DR groups.

Table 1. Effect of DR and Exercise on Disease Status (n=40)

Group	AS	AE	RS	RE
Non-Neoplasm	21	13	16	28
Nephropathy	18	9	3	0
Cardiomyopathy	0	0	3	7
Other non-neoplastic disease	1	1	0	10
Undetermined	2	3	10	11
Neoplasm	16	22	23	11
Leukemia	8	11	11	7
Pituitary adenoma	6	1	0	
Other neoplasm	2	5	11	4
Neoplasm & Non-neoplasm	3	5	1	1

AS: sedentary ad libitum group
AE: exercised ad libitum group
RS: sedentary 40% dietary restricted group
RE: exercised 40% dietary restricted group
Data obtained from Ikeno et al. (12) and McCarter et al. (10).

The Fischer 344 rat is especially prone to chronic nephropathy, the most common cause of death in this strain (1,29). Previous work from this laboratory has shown that chronic nephropathy is a major contributor to morbidity in the Fischer 344 rat (29). Subsequently, the substitution of soy for casein as the dietary protein source was shown to substantially reduce the incidence of chronic nephropathy in AL rats. Regardless of whether soy or casein is used as the protein source, DR was shown to significantly suppress the occurrence of fatal chronic nephropathy (13, 29). Exercise in the AL group was shown to attenuate the appearance of chronic nephropathy, but did not ameliorate this disease in the DR group (10,12).

With regard to cardiomyopathy, exercise enhanced the deleterious effects of age in the DR rats, alone. A higher incidence of fatal (severe) cardiomyopathy was observed in the exercised DR group than in the sedentary and exercised AL groups as well as the sedentary DR group (12). This fatal cardiomyopathy in the exercised DR group was more commonly observed at the later stages of life and may correlate with life-long, high physical activity.

Interestingly, a large fraction of both sedentary and exercised DR groups did not exhibit any obvious age-related disease; in many of these rats, the appearance of severe tissue pathology at death was absent.

Attempts have been made to quantitate the major, fatal diseases in Fischer 344 rats (viz., chronic nephropathy, cardiomyopathy, leukemia/lymphoma, and pituitary tumor) by a grading system based on the histopathological severity (1,30,31). Our group has utilized this type of quantitation to assess the overall pathophysiologic state of aged rats (32). The combined grades of severity of the four major diseases may be a good index of the "general condition" of the animals, a higher grade being indicative of a poor "general condition" (a shift from a physiological to a pathological condition). The total combined grades of severity in all

four major age-related disease at death are higher in both AL groups than in the DR groups (12, unpublished data). Results show that DR suppressed and/or delayed the occurrence of several diseases, and also retarded the progression of others. Thus, the DR animals appeared to retain their physiological integrity, while resisting the inroads of pathology. This ability to maintain their physiological integrity may lessen their susceptibility to disease.

As mentioned above, the replacement of casein with soy as the dietary protein source dramatically changed the age-related disease pattern in the sedentary AL group (12,13,29). As shown in Table 2, the soy protein diet drastically suppressed the occurrence of chronic nephropathy. Paradoxically, the change in protein source also resulted in a relatively higher incidence of neoplastic disease in the sedentary AL group (12,13,29). Moreover, in sedentary AL rats, diseases that heretofore were seldom noted as fatal are now more frequently reported as the probable cause of death. Exercise is generally shown to ameliorate disease profiles. Mild DR (10%) in combination with exercise was shown to slightly delay the occurrence of total neoplastic disease and suppress the occurrence of both multiple fatal diseases (i.e., the presence of several probable causes of death) and fatal chronic nephropathy, compared to the sedentary AL group (13). This suppressive effect of exercise with mild DR on age-related pathology may contribute to the life-extending action of exercise.

Table 2. Effect of Mild DR and Exercise on Disease Status (n=40)

Group	AS	RS	RE (10% DR)
Non-Neoplasm	12	21	14
Nephropathy	4	0	0
Impaction (Alementary tract)	0	6	1
Others	2	9	5
Undetermined	6	6	8
Neoplasm	24	18	25
Leukemia	8	8	11
Leukemia + Pituitary adenoma	2	0	0
Pituitary adenoma	3	2	4
Subcutaneous tumor	4	4	6
Others	7	4	4
Neoplasm & Non-neoplasm	4	0	1

AS: sedentary ad libitum group
RS: sedentary 40% dietary restricted group
RE: exercised 10% dietary restricted group
Data obtained from Ikeno et al. (13)

In our study (13), 40% DR suppressed and/or delayed the occurrence of total neoplastic and non-neoplastic diseases. The general pathophysiological condition of the sedentary AL groups suggests the existence of greater deterioration than in the other groups, indicating that the sedentary AL rats are more susceptible to pathology than the exercise with mild DR and the 40% DR rats. Because aging is characterized by progressive deterioration, those age-related changes render the animal more susceptible to physiological stress, disease, and ultimately death.

From this point of view, aging appears to be tightly linked to disease patterns and severity. Thus, analyses of age-associated pathologies may provide us with some insight into the possible mechanisms responsible for the effects of DR and exercise on the aging processes. DR extensively suppresses and/or delays the occurrence of age-related disease. Like DR, exercise also suppresses the incidence of age-related diseases; however, its efficacy is not as robust as that seen with DR. Both interventions, DR and exercise, prevent the age-related deterioration of the "general condition" of the host and reduce its susceptibility to physiological stress and disease. To explain the extensive beneficial effects of DR and exercise on the age-related diseases, both neoplastic and non-neoplastic, several hypotheses have been put forward (e.g., changes in energy utilization, metabolic rate, immune function, oxidative stress, etc.). These hypotheses will be discussed in detail in a later section.

Conclusions. Several conclusions can be drawn from this discussion on the modulation of age-related pathologies by DR and exercise. First, in AL rats, exercise ameliorated age-related pathology to some extent, but the beneficial effects on pathology did not remarkably alter the aging process. Second, exercise with mild (10%) DR suppressed and/or delayed the occurrence of multiple fatal diseases including chronic nephropathy. This salutary influence may contribute in part to life extension. Third, exercise with 40% DR suppressed the occurrence of total neoplastic disease. Median life span was increased, but maximum life span remained unaffected, indicating that the pathological changes due to exercise did not affect the aging processes per se. Fourth, exercise can suppress and/or delay the occurrence of age-related disease. This effect depends on the pathophysiological "general condition" of the host animal, which in turn, determines the susceptibility to physiological decline and pathological change.

Mechanisms of Aging as Probed by Dietary Restriction and Exercise

Several mechanisms have been proposed as the basis for the aging process and the action of DR on aging. These include, (i) the retardation of growth rate (14), (ii) the increase of physical activity (33,34), (iii) the protection against the development of obesity (11), (iv) the decrease of energy availability for cell proliferation (2,15), (v) the alteration of metabolic rate (17) and, finally, (vi) a reduction in oxidative stress (35-39). Interestingly, many of these hypotheses invoke, as mechanisms, the metabolic alterations observed with exercise. Therefore, it is not unreasonable to expect that exercise would provide some insight into the mechanisms of the aging processes and the action of DR. Each of the hypotheses mentioned above, as they apply to both exercise and DR, are discussed individually in the sections that follow.

Retardation of Growth Rate. The retardation of growth rate is one of the most obvious responses to DR, and in the male rat, to exercise. Because the effect is so dramatic, McCay et al. (14) proposed that slowing the growth rate is the basis for the anti-aging action of DR, while Goodrick (7) suggested that it was responsible for the beneficial effects of exercise on longevity in AL rats. However, several lines of evidence, indicate that the relationship between the retardation of growth rate (produced either by DR or by exercise) and longevity is merely correlative and not causal. First, because exercised male rats do not increase their food intake to compensate for the elevated energy expenditure, they have lower body weights than sedentary controls and resemble DR rats. In spite of the reduced availability of energy for growth, voluntary exercise did not extend maximum life span in either the AL or the DR groups in our study (10). Second, Yu et al. (5) showed that DR initiated at 6 months of age (after the rapid growth phase) is as effective as DR begun at 6 weeks of age (prior to the rapid growth phase) in extending life span, delaying age-associated physiological decline, and retarding the progression and delaying the occurrence of age-associated diseases. Finally, female, Long-Evans rats subjected to exercise did not suffer a decrease in growth rate (unlike male rats); yet, instead of shortening life span, the increased physical activity actually exerted beneficial effects on these animals (27). Therefore, from these considerations, the idea that the actions of DR and exercise resides in their ability to retard growth rate seems unlikely.

Physical Activity. DR is associated with enhanced physical activity, and it is possible that elevated physical activity itself represents a mechanism for the anti-aging action of DR (33,34). Several studies have examined the effects of exercise on the aging processes (7,9,10). Unfortunately, the results on the effects of enhanced physical activity on the maximum life span are inconsistent. Holloszy et al. (9) reported that voluntary wheel running used in combination with 8% DR does not affect maximum life span. More recently, however, McCarter et al. (24) showed that voluntary wheel running used in combination with 10% DR significantly increases maximum life span. The inconsistency of the results on exercise and maximum life span is bothersome in view of the fact that DR has consistently been shown to increase maximum life span. Until the basis for this inconsistency is determined, the view that DR and exercise alter the aging processes by increasing physical activity must remain speculative.

Adiposity. Berg and Simms (11) originally proposed that DR retarded the aging processes by reducing body fat content. Exercise has also been shown to decrease fat cell size and number (40). In an earlier study, Bertrand et al. (41) reported that body fat content does not correlate at all with length of life in AL fed, male Fisher 344 rats, but that a positive correlation exists between body fat and the length of life in DR rats (41). Furthermore, studies using Wistar rats (42) and obese (ob/ob) mice (43) did not detect any correlation between the amount of body fat and longevity. These studies render unlikely the hypothesis that the major factor responsible for the anti-aging actions of DR resides in a reduction in the amount of body fat.

Energy Availability. Weindruch and Walford proposed that DR retards the aging processes by decreasing the energy available for cell proliferation, thereby shifting the physiological state from one of growth to one of maintenance and repair (2,15). The strength of the proposal lies in the well-known anti-tumorigenic effect of DR. Moreover, a further reduction of energy availability induced by exercise in the 40% DR group elicited additional decreases in the incidence of total neoplastic disease, compared to the sedentary DR group (12). Several phenomena argue against this proposal. First, although exercise is associated with a reduced incidence of neoplastic disease, it has no effect on the incidence of age-related, non-neoplastic disease, and, in fact, actually increases the occurrence of fatal cardiomyopathy (12). Second, exercised rats with mild DR (10%) exhibits the same incidence of neoplastic disease (although with a slightly delayed occurrence) as the sedentary AL rats, even though the former treatment increases median and maximum life spans. Finally, the energy availability hypothesis, which invokes cell proliferation mechanisms, fails to explain the effects of DR on post-mitotic organs. The suppression of age-related changes by DR and exercise was not confined only to mitotic tissues; it was observed in post-mitotic tissues (brain, heart, and skeletal muscles) as well. Brain development occurs prior to the initiation of DR, thereby effectively removing any beneficial effects of DR via changes in cell proliferation. Although the brain weights of AL and DR rats were not different, DR suppressed a number of age-related physiological changes in the organ (44). Thus, although reduction of energy availability may contribute to the anti-tumor action of DR, it is unlikely that a decrease in energy availability is the essential underlying mechanism responsible for the effect of DR on the aging processes.

Immune Function. The responsiveness of the immune system progressively deteriorates with age. Both 40% DR and exercise retard the decline in immune function (45,46). Thus, because physical activity enhances immune function (47,48), and because the immune system plays an important role in tumorigenesis, the elevated physical activity associated with DR could contribute to the reduced tumor incidence observed in DR animals (10,12). Enhanced immune competence may also contribute to the delayed occurrence of neoplastic disease in both sedentary and exercised DR groups. The available evidence, however, indicates that enhanced immune function can only partially explain the extension of median life span and the decreased occurrence of neoplastic disease observed using exercise coupled with 40% DR (10,12).

First of all, exercise was found not to alter the occurrence of neoplastic disease in either the AL group or in the mild 10% DR group. This observation is surprising as exercise is expected to enhance immune function (47,48). Furthermore, it is difficult to totally credit enhanced immune function with the extensive beneficial effects of DR because both neoplastic and non-neoplastic diseases benefit by dietary intervention (3). Finally, Weindruch et al. (46) have demonstrated that the life extending effect of DR does not totally correlate with enhanced immune function when DR is initiated at various ages. In summary, although the available data suggest that enhanced immune function may play an important role in the effect of DR, and perhaps of exercise, on some age-related diseases, especially neoplastic disease, they cannot explain the extensive effect of DR and exercise on longevity and age-related non-neoplastic disease.

Metabolic Rate. A causal relation between energy metabolism and longevity was first proposed by Rubner (19) in the "rate-of-living" theory of aging. This theory is based on comparative studies in which the life span of a variety of mammals was found inversely related to their metabolic rate (i.e., length of life was determined by the intensity of metabolism). Pearl (18) refined this concept by suggesting that longevity is inversely proportional to metabolic rate normalized to body mass. Subsequently, Sacher (17) proposed that DR retards the aging processes by reducing metabolic rate. Data gained from these early studies on exercise were consistent with the "rate-of living" theory of aging (21,22).

More recently, McCarter et al. (49) directly measured oxygen consumption over a 24-hour period under usual living conditions and demonstrated that DR does not decrease the metabolic rate per unit of lean body mass (or per unit "metabolic mass"). This finding supports results from an earlier study of Masoro et al. (50), which showed that the food intake of DR rats per lean body mass does not differ from the AL controls. The results of both experiments argue against the "rate-of-living" theory of aging. The results of a recent study by McCarter et al. (10) confirm his earlier findings on metabolic rate and go substantially farther. Up to 18 months of age, exercised DR rats were found to exhibit the same metabolic rate per lean body mass as sedentary DR rats. At 24 months, however, the metabolic rate of the exercised DR rats exceeded that of the sedentary DR rats. In spite of the elevated metabolic rate shown later in life, these exercised rats enjoyed an increase in median life span and a decrease in the occurrence of neoplastic disease. The available data therefore do not support the idea that metabolic rate (per lean body mass) is the underlying regulator of the aging processes.

Oxidative Stress. The "free radical theory of aging" as originally proposed by Harman (51) has thus far weathered the test of time, and is currently the only theory of aging that has maintained wide support. The basic premise of this theory is that the rate of oxygen consumption correlates with the rate of formation of reactive oxygen species (ROS). Because these radicals are highly reactive, they may cause extensive DNA, protein, and lipid damage (52), which in turn, could lead to tissue injury, carcinogenesis (53), and aging. It is becoming increasingly clear, however, that the production of ROS represents only one facet of the cellular oxidant/antioxidant system. Although many tissues from aged animals exhibit a decline in free radical production, the generation of a whole variety of other reactive species accompanies the aging process (57). Coupled with the age-related increased oxidative state is a decrease in the cellular antioxidant concentrations (52, 54-57). Thus, shifts in the balance between oxidative stress, with its increase in ROS production, and the protective ability of cellular antioxidant defense systems, may lie at the root of the aging process and could explain the appearance of age-related diseases (57).

A novel role for oxidative stress in aging processes was recently proposed by Kristal and Yu (58), who suggest that aging is the result of interactions between glycation and Maillard reactions, which can not only create free radicals, but can augment their products. Earlier, Cerami (59) proposed that glucose is a mediator of aging because the non-enzymatic reactions of glucose with proteins and nucleic acids yield advanced glycosylation end products. These non-enzymatic reactions correlate with free radical generation and are associated with functional deterioration (60). Kristal and Yu (58) suggested a synergistic interaction among free radicals, glycation, and advanced Maillard reactions, entitling this view of aging as the "Free Radical-Glycation/Mailard Theory of Aging" (58).

One consistent finding of DR is that it ameliorates the damage caused by oxidative stress (35-38). Even with the same metabolic rate as AL rats, DR rats have a lower ROS production and higher superoxide dismutase activity within 6 weeks of DR initiation (39). DR also reduces the peroxidizability of membrane lipids and maintains the integrity of membrane structure and fluidity during aging (38). In heart mitochondria, Kim et al. (35) showed that DR lowers malondialdehyde production and elevates concentrations of antioxidant enzymes (e.g., superoxide dismutase, selenium-dependent glutathione peroxidase, and glutathione-S-transferase). As these examples point out, DR rats, which have the same metabolic rate as AL rats, are subjected to less oxidative stress than the AL animal. Another very interesting effect of DR is that it causes profound decreases in plasma glucose and insulin levels (61,62). A decline in ROS production (57) combined with a decrease in plasma glucose levels should lead to a decrease in advanced glycation end products and the associated age-related damage (58).

One common anti-aging action shared by DR and exercise is the ability to enhance antioxidant defense systems. Long-term and well-controlled exercise regimens are associated with reduced free radical injury

and an elevation in antioxidant levels in various tissues (35,36,63-67). Exercise, alone or in combination with DR, exerts beneficial effects on oxidative damage in the heart (35) and liver (36). In the latter organ, exercise suppressed microsomal ROS production, increased or maintained the levels of selected antioxidants, and exerted beneficial effects on the fluidity of microsomal and mitochondrial membranes (36). Kim et al. (35,36) suggested that the combination of exercise and DR is the most effective means of preserving membrane fluidity and suppressing microsomal ROS production. Pathological analyses support this suggestion, as the exercise with 40% DR group exhibits a marked reduction in the occurrence of total neoplastic disease compared to the sedentary 40% DR group (12). Thus, DR and exercise together, known to significantly lower plasma glucose and insulin levels (61,62,68) and enhance defense systems to protect against oxidative stress, may synergistically decrease injury from oxidative stress.

In its broadest terms, the Oxidative Stress Hypothesis of Aging proposed by Yu (57) appears to explain many, if not all, the known characteristics associated with aging and age-related disease. Oxidative stress, (i) contributes to the damage of molecular and cellular components observed with age, (ii) leads to age-related, functional deterioration, and (iii) is associated with the appearance of neoplasms. Both DR and, depending on the conditions, exercise ameliorate the deterioration and pathology associated with aging. Because both DR and exercise also attenuate oxidative stress, their anti-aging actions may depend upon their ability to maintain a proper balance between ROS production and the efficacy of the antioxidant systems.

SUMMARY

The basic mechanisms responsible for the aging processes are not fully understood. One approach to understanding these processes is to use interventions as research probes to alter their course. DR is the most effective known means of altering the aging process and the most frequently used intervention of gerontologists. DR has consistently been shown to increase life span, decrease the frequency of occurrence and severity of age-related pathology, and retard much of the physiological decline associated with aging. Unfortunately, the mechanisms by which DR acts on the aging process are unknown and remain almost as elusive as those of aging itself.

Exercise, both alone and in concert with DR, has been used to probe the aging processes as well as the mechanisms responsible for the anti-aging action of DR. Much of the available data suggest that exercise, when applied alone, does not increase maximum life span, and therefore may not retard aging per se. On the other hand, exercise, applied either alone or in concert with DR, exerts beneficial effects on the median life span. It has not yet been ascertained whether the anti-aging action of DR is based, in part, on the increase in physical activity, as seen with exercise.

The beneficial effects of exercise are especially evident when the optimal level of exercise is applied in conjunction with DR: used in concert with 40% DR it suppresses the appearance of neoplastic disease which, in turn, leads to an extension of median life span. Pathological analyses of rats subjected to exercise lags behind those performed on DR rats. This is unfortunate because the development of pathological patterns differ between DR and exercise, and therefore may hold information concerning the mechanisms underlying each or both of these aging interventions.

What is clear, however, is that DR alters the occurrence and severity of several neoplastic and non-neoplastic diseases, and that exercise has similar effects when coupled with mild DR and has additional anti-tumor action when coupled with 40% DR (12,13). A more vigorous pathological analysis of exercised rats would aid in assessment of the interactions between DR and exercise and in each of their effects on the aging processes.

Despite the relative paucity of data on the effects of exercise on aging and its pathologies, the results from available studies have been helpful in assessing the validity of the hypotheses proposed to explain the action of DR on aging. For example, experiments utilizing exercise have produced results that are inconsistent with several hypotheses, including "retardation of growth rate," "energy availability," and the "metabolic rate theory." In addition, the idea that DR acts by enhancing the immune function, although attractive as it may be when associated with a decrease in neoplastic disease, does not address the effects of DR and exercise on non-neoplastic diseases. To date, only the "oxidative stress theory of aging" (57) can account for most, if not all, of the data gathered using DR, exercise, and pathological analysis. On one hand, exercise is associated with enhancing the immune system and the defense systems' ability to protect against oxidative damage. On the other hand, the pathologies associated with aging, both neoplastic as well as non-neoplastic, are reduced by both DR and exercise. This conclusion suggests that examination of the effects of DR and exercise on cellular antioxidant systems and on the age-related changes in these systems may prove fruitful in delineating the mechanism by which DR alters the aging processes and indeed in elucidating the basis for the aging processes themselves.

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